Apr 16, 2025

DNA quantification with PicoGreen

Forked from DNA quantification with PicoGreen

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpdbr1gzp/v1

Alexander Eiler[1], Eivind Stensrud[2,1]

[1]University of Oslo; [2]eDNA solutions AB

eDNAsolutions
Tech. support phone: **+46 (704) 139-481** email: **eivind@ednasolutions.se**

Eivind Stensrud
eDNA solutions AB

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.dm6gpdbr1gzp/v1

Protocol Citation: Alexander Eiler, Eivind Stensrud 2025. DNA quantification with PicoGreen. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpdbr1gzp/v1

Protocol status: Working
We use this protocol and it's working

Created: April 16, 2025

Last Modified: April 16, 2025

Protocol Integer ID: 126785

Keywords: quantification, DNA, Picogreen, dna quantification with picogreen, dna quantification, selective for dsdna, dsdna, asymmetrical cyanine dye, ssdna, free dye, sensitive than uv absorbance method, picogreen, rna, uv absorbance method

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

The Quant-iT™ PicoGreen® dsDNA reagent is a proprietary, asymmetrical cyanine dye. Free dye does not fluoresce, but upon binding to dsDNA it exhibits a >1000-fold fluorescence enhancement. PicoGreen is 10,000-fold more sensitive than UV absorbance methods, and highly selective for dsDNA over ssDNA and RNA.
For more information see: https://www.thermofisher.com/order/catalog/product/P7589

Materials

MATERIALS

Quant-iT™ PicoGreen® dsDNA Assay Kit **Life Technologies Catalog #**P7589

Before start

Take out PicoGreen (supplied in the PicoGreen kit) from fridge to thaw, before starting.
Protect it from light.

PicoGreen is solid at 4°C and thaws at room temperature. The reagent is light sensitive and should be kept wrapped in foil both while thawing and in the diluted stage. Recommeded to thaw PicoGreen in a closed drawer 30 minutes before use.

Before starting

1 **Prepare TE**

Calculate the total volume of 1xTE needed:

- 200 µl per sample
- 100 µl per standard
- Total of 3-400 µl for the DNA standard dilution (depending on the assay you use, see step 2)
- Make 15 % extra to account for pipetting losses!
- E. g. for medium range: 20 wells (10 samples in duplicates) + 2*4 standards (4 standards in duplicate) + DNA standard the amount is 10*2*200 + 2*4*100 + 400 = 5200 µl → make 5980 µl

Dilute from 20xTE stock (supplied in the PicoGreen kit).

- Volume of 20xTE = final volume/20;
- Volume of water = final volume – volume of 20*TE
- E.g. To make 5980 µl: mix 299 µl of 20*TE with 5681 µl of water.

2 **Prepare DNA standard**

We recommend to use the **middle range** for both DNA extract and PCR products. Be careful to work with high concentration DNA in Pre-PCR, please add DNA standard in Post-PCR.

Dilute 100 µg/mL **DNA standard** (supplied in the PicoGreen kit) to working dilution.

- For high range prepare 2 ng/µl: mix 6 µl of DNA stock with 294 µl of 1*TE
- For **middle range** prepare 0.5 ng/µl: mix 2 µl of DNA stock with 398 µl of 1*TE
- For high sensitivity prepare 0.05 ng/µl: mix first 2 µl of DNA stock with 18 µl of 1*TE (working solution), then mix 2 µl of the working solution with 398 µl of 1*TE

3 **Prepare PicoGreen**

Remember to thaw the PicoGreen 30 minutes before usage.

Try to work as dark as possible when using PicoGreen.

Wrap both the stock, and diluted PicoGreen in foil.

Calculate the total volume of 1:200 diluted PicoGreen reagent needed.

- For each standard and each unknown sample, a volume of 100 µL will be needed = total number of samples (standard + unknowns) x100 µl + 10% extra for pipetting losses.
- e.g. For standard series of 8 and 20 samples (=28 total) prepare 28*100 µl =2800 µl: → make 3080 µl

Dilute PicoGreen reagent with 1*TE.

- Volume of PicoGreen = final volume/200;
- Volume of TE = final volume – volume of PicoGreen
- e.g. Mix 15,4 µl of PicoGreen with 3064,6 µl of 1*TE

KEEP diluted PicoGreen IN THE DARK UNTIL USE!!!

4 **Prepare plate - add standards**

- Use **NUNC 96-well flat bottom black** plates. Cover the plate with aluminium foil.
- Mark samples needed to be measured on the lid and also diagram your plate layout in the table as follow (When possible, measure samples at least in duplicates).
- Feel free to use the template shared here: https://docs.google.com/spreadsheets/d/1kCx8UDrrJ6XsSPVBYYeuk6XgoKGtnrsapPWuVBYbt_U/edit?usp=sharing
- Plan the standard curve in the microtiter plate.
- Usage of multipipette and reagent reservoir is recommended.

Table 1 High range standard curve: for 2ul samples, test limit is **0 to 100** ng/ul - for higher concentration dilute your samples.

	Plate Well	volume of TE (uL)	volume (uL) of **2ug/mL** stock DNA	volume of diluted picog dye (uL)	final DNA concentration in assay (ng/mL)	total DNA in well (ng)
	A1 & A2	0.00	100.00	100	1000	200
	B1 & B2	90.00	10.00	100	100	20
	C1 & C2	99.00	1.00	100	10	2
	D1 & D2	100.00	0	100	0	0

Table 2 **Medium range** standard curve: for 2ul samples, test limit is **0 to 25** ng/ul. Recommended standard curve.

	Plate Well	volume of TE (uL)	volume (uL) of **0.5ug/mL** stock DNA	volume of diluted pg reagent (uL)	final DNA concentration in assay (ng/mL)	total DNA in well (ng)
	A1 & A2	0.00	100.00	100	250	50

	B1 & B2	90.00	10.00	100	25	5
	C1 & C2	99.00	1.00	100	2.5	0.5
	D1 & D2	100.00	0	100	0	0

Table 3 Low range standard curve: for 2ul samples, test limit is **0 to 2.5** ng/ul

	Plate Well	volume of TE (uL)	volume (uL) of **0.05ug/mL** stock DNA	volume of diluted pg reagent (uL)	final DNA concentration in assay (ng/mL)	total DNA in well (ng)
	A1 & A2	0.00	100.00	100	25	5
	B1 & B2	90.00	10.00	100	2.5	0.5
	C1 & C2	99.00	1.00	100	0.25	0.05
	D1 & D2	100.00	0	100	0	0

5 **Prepare plate - add TE**

- Add 1*TE to each standard well according to table 1-3.
- Add 95-98 µl of 1*TE to each sample well. Prepare 2 wells for each sample (duplicates). I.e. if you add 2 µl of sample add 98 µl of 1*TE in the case of 5 µl sample add 95 µl 1*TE (see point 6).

6 **Prepare plate - add DNA samples**

- Add 2-5 µl of sample to each sample well.

7 **Prepare plate - add DNA standard**

- Add diluted DNA standard according to Table 1-3.

8 **Prepare plate - add PicoGreen**
Remember to work with reduced light.

- Add 100 µl of diluted PicoGreen to each of the wells (both sample and standard wells) and mix by pipetting 5-10 times.

9 Incubate in room temperature in dark for at least 5 minutes and measure with the plate reader (ask for help using the plate reader if using it for the first time).
00:05:00

10 **Settings for BioTek Synergy 2 plate reader.**
1. When plate(s) are ready, turn on the power supply, then switch on the BioTek Synergy 2 plate reader (5 min before use), then turn on the computer.
2. Log into the computer.
3. Open the program Gen5 1.11 (top right corner on the screen).
4. Open an the protocol called DNA_quantification_AQUA.

If there is no excisting protocol, following these steps:
1. To make sure, select Procedure, select Plate Type: Default: Costar 96 black opague.
2. Detection Method: Fluorescence, Read Type: Endpoint, Read Speed: Normal, sensitivity: 65%.
3. Filter Sets:
Excitation: ~485/20nm, Emission:~528/20 nm.
Optics Position: Top, sensitivity: 65, Top Probe Vertical Offset: 8,0 mm.
4. Incubator Off.
5. Save Protocol.

Open experiment using the newly created Protocol:
1. Save the experiment, with a filename consisting of you surname and date.
2. Ensure the Plate layout is correct.
3. Place plate on tray with the lid on. Wait until the machine is ready.
4. When ready, take of lid, and press ok/insert.

Results and analysis:
1. Save the result on the computer.
2. Open the readings in Excel, and transfer to a USB stick.
3. Transfer to your local computer.

11 **To calculate your DNA concentration:**
Feel free to use the Google sheet template:
https://docs.google.com/spreadsheets/d/1kCx8UDrrJ6XsSPVBYYeuk6XgoKGtnrsapPWuVBYbt_U/edit?usp=sharing
1. Create a calibration curve in excel or similar from the standards (x axis: measured fluorescens, y axis: total DNA in standard wells in ng from corresponding tables in step 4).
2. Use the equation of the fitted trendline (excel) to calculate the amount of DNA (ng) in your samples.
3. To calculate the DNA concentration of your samples (ng/µl), divide the calculated amount of DNA (ng) by the volume of DNA (µl) that you added in step 6 (2-5 µl).